

Rapid evolution of thermal tolerance in the water flea *Daphnia*

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Global climate is changing rapidly, and the degree to which natural populations respond genetically to these changes is key to predicting ecological responses^{1–3}. So far, no study has documented evolutionary changes in the thermal tolerance of natural populations as a response to recent temperature increase. Here, we demonstrate genetic change in the capacity of the water flea *Daphnia* to tolerate higher temperatures using both a selection experiment and the reconstruction of evolution over a period of forty years derived from a layered dormant egg bank. We observed a genetic increase in thermal tolerance in response to a two-year ambient +4 °C selection treatment and in the genotypes of natural populations from the 1960s and 2000s hatched from lake sediments. This demonstrates that natural populations have evolved increased tolerance to higher temperatures, probably associated with the increased frequency of heat waves over the past decades, and possess the capacity to evolve increased tolerance to future warming.

Average global temperatures have substantially increased during the past 50 years and are expected to increase 0.4 to 4.8 °C in the next hundred years⁴. Populations, communities and ecosystems have responded to these changes in various ways, including shifts in species ranges, and in flowering and migration times^{1,5}. Although natural populations may adapt genetically to climate change, their ability to evolve fast enough to keep pace with observed rates of climate change is debated^{3,5–7}. Understanding whether evolution can mediate species responses to climate change is fundamental because the ability to genetically track changes in climate can reduce extinction rates. In addition, local adaptation to changing temperatures can also modulate the relative importance of local and regional processes, as it changes competitive interactions between residents and immigrants, potentially reducing establishment success of immigrants^{2,8}.

Climate change and associated increases in frequency of heat waves⁹ induce physiological stress owing to increased metabolic rates and oxygen demand^{10,11}. Thermal performance curves and measures of heat tolerance have been used to predict organisms' responses to climate change^{10,12,13}. A widely used index of heat tolerance is the critical thermal maximum (CT_{Max}), which is the upper temperature at which animals lose motor function. CT_{Max} is heritable in the fruit fly *Drosophila*, is a measure of performance under field conditions¹⁴, and is informative for climate change responses in several species^{15,16}. Yet, no study of natural populations

has so far documented a genetic increase in CT_{Max} in response to recent climate change.

Here, we first use an experimental evolution approach to test whether a natural population of the water flea *Daphnia magna* Straus (1820), a key zooplankton organism in shallow freshwater ecosystems¹⁷, harbours sufficient genetic variation in CT_{Max} to adapt genetically to increased water temperature. *D. magna* typically inhabits shallow lakes and ponds, which are vulnerable to changes in temperature. Next, we use resurrection ecology to quantify evolutionary changes in CT_{Max}. Resurrection ecology uses the layered dormant propagule bank in sediments as a genetic archive that allows the reconstruction of microevolution in a given population through time^{18–20}. Lake sediments are typically layered and contain dormant stages of zooplankton that represent snapshots of the population through time. By hatching genotypes produced during these different time periods we can study the genetic changes that occurred in the population. *D. magna* were resurrected from a half-century-old egg bank (1955–1965) of a natural population and their CT_{Max} values were compared with those of more recent (1995–2005) *D. magna* from the same pond. As such, we were able to determine realized shifts in CT_{Max} in response to recent climate change. This approach has successfully been applied to reconstruct evolution in phenology and physiology in response to climate change in the plant *Brassica rapa*^{21,22}.

In the experimental evolution trial, we exposed a genetically diverse population of *D. magna* (150 clones), to either ambient temperature or to an ambient +4 °C temperature treatment in outdoor tanks (mesocosms) that mimicked small ponds. After two years, sediments were collected from two heated- and two ambient-temperature mesocosms and twenty genotypes were hatched from dormant eggs in these sediments—that is, probing post-selection populations (see Supplementary Information I.A for more details). The resulting clones were scored for CT_{Max}. Results confirmed that natural *Daphnia* populations have the evolutionary potential to respond genetically to a strong but environmentally relevant temperature increase. Clones from heated mesocosms had, on average, a 3.6 °C higher CT_{Max} than clones from the ambient-temperature mesocosms (temperature treatment effect $P < 0.001$; Table 1 and Fig. 1a). The difference in CT_{Max} between the populations from the two temperature treatments matched the +4 °C temperature difference between the two selection regimes.

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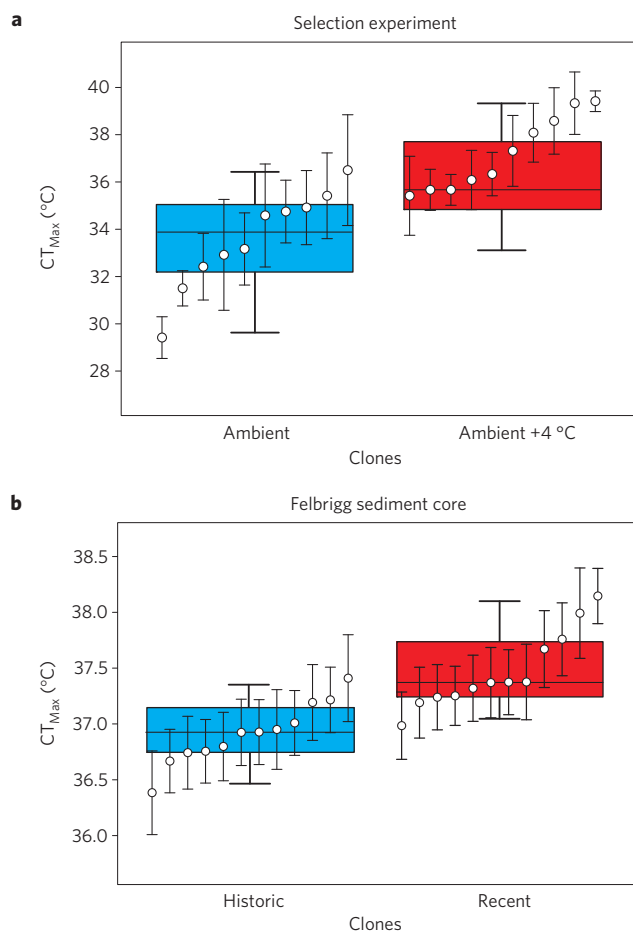


Figure 1 | Average critical thermal maxima for *Daphnia* clones from the selection experiment and from Felbrigg Hall Lake. **a, Average CT_{Max} values for the 20 *D. magna* clones hatched from sediment from the selection experiment, grouped by selection temperature: 'ambient' (blue boxplot) or 'ambient +4 °C' (red boxplot). **b**, Average CT_{Max} values of the 24 *D. magna* clones hatched from the Felbrigg Hall Lake sediment cores. Historic = clones from sediment in Felbrigg Hall Lake dated to 1955–1965 (blue boxplot); recent = clones from sediment in Felbrigg Hall Lake dated to 1995–2005 (red boxplot). In each plot clones are ordered within population according to increasing CT_{Max} . Means are given ± 1 s.e.m. Boxplots represent the median, 25th percentile and the 75th percentile, error bars indicate 10th and 90th percentile in CT_{Max} per population.**

The resurrection ecology study used two sediment cores from Felbrigg Hall Lake, a shallow (<1 m) lake in Norfolk, UK. Twelve genotypes were hatched from historic (1955–1965) and twelve from recent (1995–2005) sediment layers (see Supplementary Information I.B). Clones hatched from recent sediments had a significantly higher CT_{Max} than clones hatched from eggs formed forty years earlier ($P < 0.001$; Table 2 and Fig. 1b). Heritabilities for CT_{Max} were 0.24 and 0.33 for the set of clones hatched from the historic and recent sediment layers, respectively, suggesting evolutionary potential for this trait in both populations separated in time. Population genetic analysis of 43 microsatellite markers confirmed genetic continuity of the natural population of Felbrigg Hall Lake through time (F_{ST} value quantifying the among-group component of genetic variation in allele frequencies = 0.022).

Body size at maturity did not differ significantly between clones from recent and historical sediment layers ($F_{1/23.2} = 0.12$, $P = 0.729$), but size had a significant negative effect on CT_{Max} ($P < 0.01$, Table 2 and Fig. 2). Although there was no significant size by population

Table 1 | Results of the linear mixed model showing the effects of selection background (temperature treatment) of *Daphnia* clones on their CT_{Max} .

Fixed effects	d.f.	F value	P	
Temperature treatment	1, 20	19.48	0.0003	
Mesocosm (Temp. treatment)	2, 20	0.53	0.5973	
Day	1, 100	5.03	0.0271	
Random effects	Estimate	s.e.m.	Z value	P
Clone (Temp. treat. *mesocosm)	0.885	0.9712	0.91	0.1810

Significance of random effects is tested by approximate Wald tests. For fixed effects, significance is tested using approximate F -tests. The variable 'day' refers to the day (1 or 2) on which CT_{Max} was scored. Analyses of the data for each day separately (see Supplementary Information II) revealed that the temperature treatment is consistently significant across both days. Clones were nested in mesocosm, which in turn were nested in temperature treatment.

Table 2 | Results of the linear mixed model showing the effects of selection background (sediment layer) in the resurrection ecology analysis of *Daphnia* clones on their CT_{Max} .

Fixed effects	d.f.	F value	P	
Sediment layer	1, 23.6	18.97	0.0002	
Body size (mm)	1, 182	8.93	0.0032	
Random effects	Estimate	s.e.m.	Z value	P
Clone (sediment layer)	0.073	0.029	2.55	0.0055
Day	0.015	0.014	1.06	0.1450

Significance of random effects is tested by approximate Wald tests. For fixed effects, significance is tested using approximate F -tests. The table shows differences between clones hatched from sediment layers from 1955–1965 and 1995–2005 in Felbrigg Hall Lake. The nonsignificant interaction effect of body size and sediment layer ($F_{1/179} = 2.71$, $P = 0.10$) was removed from the model. Clones were nested in sediment layer.

(historic versus recent) interaction effect on CT_{Max} , there was a trend for a stronger effect of body size on CT_{Max} in the recent ($\beta_1 = -1.08$, $F_{1/83} = 13.52$, $P < 0.05$) compared with the historic ($\beta_1 = -0.35$, $F_{1/83} = 1.50$, $P = 0.22$) population (Fig. 2 and Supplementary Fig. 1, see Supplementary Information II for more details).

Our results clearly show the potential of natural populations of the water flea *Daphnia* to evolve higher heat tolerances. Natural populations of many organisms exhibit heat tolerances or temperature performances that generally, but not always, match local temperature regimes^{10,12}. Among *Daphnia* populations from different latitudes, no differences were observed in temperature performance curves as measured in life-table analyses²³, whereas a positive relationship was observed between heat tolerance and the average temperature of the warmest month²⁴. Genetic adaptation to local temperature has also been revealed in different natural populations of the reef coral *Acropora hyacinthus*²⁵ at relatively small spatial scales. In other systems, phenology has been shown to evolve, as in the case of egg hatching date in the winter moth *Operophtera brumata*²⁶. However, the present study is the first to demonstrate both the capacity for rapid evolution of thermal tolerance and actual genetic change over recent decades associated with climate change in natural populations.

Specifically, the observed changes in genotypic CT_{Max} values reported here show that natural *Daphnia* populations have the evolutionary potential to genetically track an acute change in average temperature of at least 4 °C and can do so in two years or less. In addition, our resurrection ecology analysis shows that a natural *Daphnia* population has already genetically tracked changes in temperature over 40 years. Temperature records are consistent with a scenario of selection for increased thermal tolerance. Norfolk, where the lake is situated, has experienced an increase in average temperature of 1.15 °C and a threefold increase in the number of

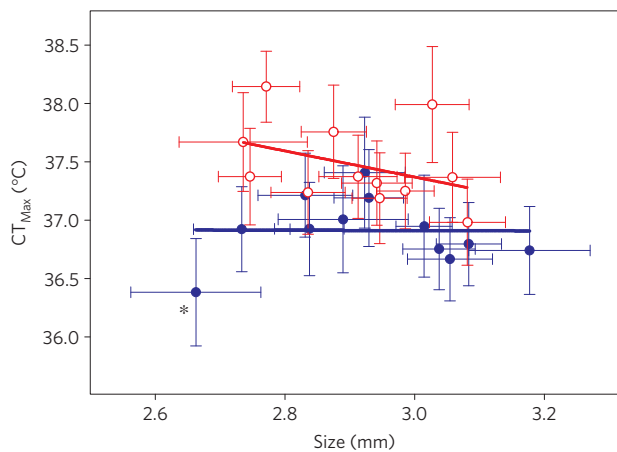


Figure 2 | Average critical thermal maxima plotted against average adult size for clones from Felbrigg Hall Lake. CT_{Max} values ± 1 s.e.m. and average adult size ± 1 s.e.m. are shown per clone and grouped by sediment layer. Blue filled circles = clones from sediment in Felbrigg Hall Lake dated to 1955–1965; red open circles = clones from sediment in Felbrigg Hall Lake dated to 1995–2005. Solid lines show linear regressions for the historic (blue line: $\beta_1 = -0.35$, $F_{1/83} = 1.50$, $P = 0.22$) and recent (red line: $\beta_1 = -1.08$, $F_{1/83} = 13.52$, $P < 0.05$) populations. The clone marked by an asterisk is clone H8 (see Supplementary Information II).

heat waves (average temperature $> 30^\circ\text{C}$ for at least two consecutive days) in the 2000s compared with the 1960s²⁷, a trend that will probably continue in the future⁴.

Our study is the first to tap into the genetic archive provided by the egg bank of a natural population to reconstruct genetic adaptation to increasing temperatures in *Daphnia* over several decades. Resurrection ecology has been used successfully in *Daphnia* to document evolution of increased cyanotoxin tolerance²⁸ and antipredator behaviour¹⁹ as well as host–parasite co-evolutionary dynamics²⁰. Moreover, it has been successfully used to reconstruct evolution in plants²¹. By using resurrection ecology to study rapid evolution in nature, we have circumvented potential artefacts associated with experimental evolution trials, such as the unnatural pattern of the imposed acute increase in temperature²⁹. The results of our two approaches reinforce each other: while the experimental evolution probes for evolutionary potential, our analysis of the sedimentary archive quantifies observed and recent evolutionary change in nature. Furthermore, although our resurrection ecology study documents a response over the past 40 years, our experimental evolution trial shows that contemporary populations harbour genetic variation that may fuel further responses. Moreover, the response we observed in the mesocosm experiment is probably adaptive. In earlier research comparing water flea populations exposed to ambient temperature with those in the $+4^\circ\text{C}$ temperature treatment, we showed an increased capacity to suppress establishment success of immigrant genotypes that were derived from a lower latitude at which a warmer climate (approx. ambient $+4^\circ\text{C}$) prevails³⁰. The observation that our two approaches (experimental evolution and resurrection ecology) lead to very similar genetically based changes in thermal tolerance adds confidence to our interpretation that the responses observed in the resurrection ecology study reflect adaptive responses to climate change.

The fact that CT_{Max} specifically measures tolerance to extreme temperatures¹⁵ means the observed evolutionary changes in CT_{Max} were probably driven by changes in maximum temperatures associated with heat waves rather than changes in average temperatures¹⁶. We also observed that CT_{Max} is related to body

size in the resurrection ecology study, with smaller animals having a higher CT_{Max} than larger animals. This size dependence is consistent with the interpretation that there may be a constraint imposed by the increased oxygen demand of body tissues at higher temperatures¹¹. Our observations suggest that the animals do not respond to climate change by becoming smaller but by increasing size-dependent CT_{Max} .

There is an increasing number of studies that report rapid evolutionary responses to environmental change in natural populations^{19,31}, potentially fuelling eco-evolutionary interactions that may impact biological responses to (human-induced) environmental change^{30–32}. Our observation that natural populations are capable of genetically tracking increases in temperature does not necessarily imply that those populations are physiologically buffered from climate change³³. Rather, it implies that their responses are modified. The degree to which populations may adapt to climate change fundamentally alters predictions and projected future occurrences of species^{2,8}. Genetic adaptation of resident populations may reduce establishment success of immigrant genotypes³⁰ and species² and may thus influence the relative importance of local processes compared with migration as drivers of species and community responses to climate change^{2,34}. Evolutionary potential and evolutionary change should not be ignored in attempts to predict responses of populations, communities and ecosystems to climate change^{2,34}.

Methods

Methods and any associated references are available in the [online version of the paper](#).

Received 14 November 2014; accepted 25 March 2015; published online 27 April 2015

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Acknowledgements

A.N.G. is supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT Vlaanderen). The mesocosm experiment at Liverpool was supported by the EU IP project EUROLIMPACS (EU Contract No. GOCE-CT-2003-505540), and the overall study was supported by KU Leuven Research Fund Excellence Center financing PF/2010/07 and by ERA-Net BiodivERsA project TIPPINGPOND, nationally funded by Belspo. T. H. Haileselasie kindly helped with the analysis of genetic differentiation using microsatellite markers. We thank the National Trust for access to Felbrigg Hall Lake and thanks also to R. Huey and T. Ceulemans for valuable comments on an earlier version of the manuscript.

Author contributions

L.D.M. and A.N.G. designed the overall research project. B.M., D.A. and H.F. designed the climate experiment and W.V.D. and L.D.M. developed the evolutionary selection module. W.V.D. and H.F. carried out the selection experiment. T.A.D. and C.D.S. took and analysed the sediment cores and carried out palaeolimnological analyses. A.N.G., L.D.M. and J.V. developed the resurrection ecology experiment. A.N.G. hatched the eggs and carried out the standardized CT_{max} experiments. A.N.G. together with J.V. and B.V. carried out the statistical analyses with input from L.D.M. A.N.G. and L.D.M. wrote the manuscript. All authors contributed to editing the manuscript.

Additional information

Supplementary information is available in the [online version of the paper](#). Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.N.G.

Competing financial interests

The authors declare no competing financial interests.

Methods

In the experimental evolution approach we capitalized on a large-scale thermal selection experiment simulating global warming (ambient treatment and +4 °C treatment) under semi-natural field conditions that was carried out at the outdoor experimental area of the University of Liverpool (UK). After two years, we collected the top sediment layer of heated and ambient treatment mesocosms. After extraction of the dormant eggs, ten genotypes per treatment were hatched and cultured as clonal lineages (see Supplementary Information I.A for more details).

In the resurrection ecology analysis, *D. magna* dormant eggs were obtained from sediment cores of Felbrigg Hall Lake (North Norfolk, UK 0.9 m average depth). Twelve clones were hatched from historic (1955–1965) and twelve from recent (1995–2005) sediment layers. To assess genetic continuity we tested for genetic differentiation between the historic and recent hatchlings using 43 microsatellite markers. Low F_{ST} values reflect genetic continuity of the *D. magna* population inhabiting Felbrigg Hall Lake through time (see Supplementary Information I.B for more details).

To minimize interference from maternal effects, all clones were cultured for at least two generations under standardized experimental conditions. For the experimental evolution approach CT_{Max} was measured in six independent replicates of each clone. For the resurrection ecology analysis CT_{Max} was scored for eight independent replicates of each clone, each replicate consisting of measures on six to ten different adult individuals. Body size was measured for three individuals from each replicate. Temperature was gradually increased from 20 °C to 45 °C in steps of 1 °C per 20 s. CT_{Max} was assessed as the temperature at which individuals lost their motor function and sank to the bottom of the tube (see Supplementary Information I.C for more details).

Data were analysed using linear mixed models, PROC MIXED in SAS v.9.3 (SAS Institute). We chose the maximum likelihood (ML) estimation method for our models. ‘Variance component’ was chosen for the covariance structure. For inference concerning the covariance parameters (random variables) we used the Wald Z statistic. For fixed effects, significance is tested using approximate F-tests. Degrees of freedom calculations for fixed effects were corrected by specifying the Kenward–Roger option (see Supplementary Information I.D for more details).